Comprehensive Analysis of Human Leukocyte Antigen Class I Alleles and Cervical Neoplasia in 3 Epidemiologic Studies

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To comprehensively explore the relationship between human leukocyte antigen (HLA) class I alleles and cervical neoplasia, a subset of participants from 3 large US and Costa Rican cervix studies were typed for HLA class I alleles. Study subjects were women with cervical cancer or high-grade squamous epithelial lesions (HSILs; $n = 365$) or low-grade squamous epithelial lesions (LSILs; $n = 275$) or who were cytologically normal (control subjects; $n = 681$). Allele-disease associations were assessed by logistic regression analysis. Consistent associations across all studies were observed for *HLA-CW*0202* with a combined odds ratio of 0.53 (95% confidence interval [CI], 0.29–0.89) for cancer or HSILs and 0.58 (95% CI, 0.37–1.04) for LSILs, compared with control subjects and adjusted for study. This finding supports the hypothesis that a single allele may be sufficient to confer protection against cervical neoplasia. Given the relationship between HLA-C and its receptors on natural killer (NK) cells, a role is proposed for NK function in human papillomavirus infection and cervical neoplasia.

HLA alleles involved in presenting foreign antigens to immune cells are important in host immune responses to viral and other pathogens. Among the most polymorphic human genes [1], HLA polymorphisms result in variations of the peptide-binding cleft and influence specificity of the antigens bound and presented to T cells. Class I HLA molecules (HLA-A, -B, and -C) present foreign antigens to CD8 cytotoxic T lymphocytes (CTL), and class II molecules (HLA-DR, -DQ, and -DP) present antigenic peptides to CD4 T helper cells [2, 3]. Although the importance of HLA class II genes in cervical neoplasia pathogenesis has been demonstrated over the past decade [4–11], class I allele associations with cervical neoplasia have not been widely documented. However, CTL responses to viral infections (specifically to human papillomavirus [HPV] infection) have been well documented [12, 13]. In addition, downregulation, as well as complete loss of class I antigen expression, has been reported in cervical cancer and its immediate precursors. Such alterations in class I antigen expression enable HPV-infected cells to escape detection by the immune system by becoming nonimmunogenic [14–17]. Although the role that HLA class I molecules play in cervical neoplasia is well established, the importance of individual HLA class I alleles has not been fully explored.

Development of high-resolution genotyping allowed us to complete HLA typing for 3 large cervical neoplasia studies in the United States and Costa Rica. To identify HLA alleles of importance in cervical neoplasia, we typed HLA class I and class II alleles from the cervical neoplasia studies of 2 ethnically distinct populations. HLA class II allele findings have been published from our 24,000-woman cohort in Portland, Oregon [18], and from our 10,077-woman cohort in Guanacaste, Costa

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Table 1. Description of final study populations typed for HLA class I alleles.

Study population	Study type	Cancer or HSILs[a]	LSILs[b]	Control subjects[c]	Total
Guanacaste, Costa Rica	Population-based cohort	124	79	118	321
Portland, Oregon	Kaiser Permanente cohort	124	196	350	670
Eastern United States	Six-center case-control	117	—	213	330
Total		365	275	681	1321

[a] The Costa Rican and Eastern US cohorts included patients with both cancer and high-grade squamous epithelial lesions (HSILs); patients in the Portland study had HSILs only.

[b] The Costa Rican and Portland cohorts had low-grade squamous epithelial lesions (LSILs); there were no cases of LSILs in the eastern US study. In Costa Rica, patients with LSILs were population based; in Portland, patients with LSILs were selected on the basis of human papillomavirus (HPV) infection (99 patients were HPV-16 positive, and 97 patients were positive for other HPV types).

[c] Costa Rica and eastern US control subjects were population based; Portland control subjects were selected on the basis of HPV status (159 were HPV-16 positive, and 191 were HPV negative).

Rica [19]. As in the HLA class II analyses, the extreme polymorphism of HLA class I alleles and their fairly even distribution result in low frequencies of individual alleles, making single allele-disease associations difficult to observe. Here, we present study-specific and combined results for HLA class I allele–disease associations from all 3 studies. We examined HLA class I involvement in the development of cervical neoplasia and identified consistent associations in the distinct study populations.

Subjects and Methods

Study population. Participants were selected from 3 studies sponsored by the National Cancer Institute: a 10,077 woman population-based cohort in Guanacaste, Costa Rica [20]; a 24,000 woman cohort in Portland, Oregon [18]; and a 750 woman multicenter study of histologic subtypes of cervical neoplasia in the eastern United States [21]. Details of the study designs have been described elsewhere [18, 20, 21]. The Costa Rican cohort is an ethnically admixed population, whereas the 2 US study groups are predominantly white.

In the Costa Rican cohort of women diagnosed with cancer ($n = 40$), high-grade squamous intraepithelial lesions (HSILs; $n = 130$), and low-grade squamous intraepithelial lesions (LSILs; $n = 106$) and in population control subjects ($n = 250$), a subset of 24 (60%) patients with cancer, 100 (77%) patients with HSILs, 79 (75%) patients with LSILs, and 118 (47%) control subjects were typed for HLA class I alleles. In the Portland cohort of women with HSILs ($n = 141$) and LSILs ($n = 212$) and cytologically normal control subjects ($n = 368$), a subset of 124 (88%) women with HSILs, 196 (92%) women with LSILs (99 HPV-16 positive and 97 HPV negative), and 350 (95%) cytologically normal control subjects (HPV-16–positive women were oversampled) were typed for HLA class I alleles. In the eastern US study of 234 in situ and invasive squamous cell carcinomas and 307 population-based control subjects, a subset of 117 (50%) women with cancer and 213 (69%) cytologically normal population-based control subjects were typed for HLA class I alleles. The 166 adenocarcinomas that were part of the eastern US study were not included in the present analysis.

Final analytic groups. For the present study, 1321 women were typed for HLA class I alleles, including 321 from the Costa Rican cohort, 670 from the Portland cohort, and 330 from the eastern US study. Our final analytic group consisted of 365 women with cancer or HSILs, 275 with LSILs, and 681 with normal cytologic test results (control subjects; table 1).

HLA testing. HLA class I loci were molecularly typed with DNA extracted from buffy coat [20, 21] or cervicovaginal lavage samples [18] collected from each participant. HLA class I genes were typed by using polymerase chain reaction (PCR) and single-stranded oligonucleotide probe-based protocols developed by the 13th International Histocompatibility Workshop (http://www.ihwg.org/protocols/protocol.htm).

HPV testing. Cervicovaginal samples were tested by PCR for HPV DNA, as described elsewhere [18, 20, 21]. In the Portland cohort, HPV was typed with MY09/11 consensus primers via dot blot. In the eastern US case-control study, HPV was also typed with MY09/11 consensus primers, but by strip technology. In the Costa Rican cohort, HPV typing was done by both PCR (with MY09/11) and the hybrid-capture tube test.

Statistical methods. A case-control analysis was conducted in which patients with cancer or HSILs and patients with LSILs were compared with population control subjects. We combined women with invasive cancer with our HSIL group for the final analysis. However, because HSILs comprised cervical intraepithelial lesion (CIN) 3 as well as CIN2, we also conducted separate analyses for CIN3, CIN2, and cancer. Because there was no notable difference between the independent groups with regard to allele frequency, we combined HSILs and cancer for the final analysis.

HLA-A, -B, and -CW allele frequencies were initially calculated, and statistical differences between case patients and control subjects were identified by χ^2 test for significance or by Fisher's exact test when there were <5 subjects per cell. Further analyses for alleles found to be significantly different between case and control groups were conducted. For these alleles, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to determine the magnitude and statistical significance of associations [22, 23]. Although we used logistic regression analysis to examine allele-disease associations, while adjusting for other alleles whose frequencies were significantly different between case patients and control subjects (e.g., where all alleles undergoing further analyses are placed in the same regression model), these adjustments did not alter the results. Thus, for study-specific ORs, unadjusted risk estimates are shown. For combined analyses where data for case patients and control subjects from the 3 studies were merged to obtain an overall OR, the es-

Table 2. Study control subjects possessing specified HLA class I alleles.

	Costa Rica (n = 118)[a]		Eastern United States (n = 213)[b]		Portland, Oregon (n = 350)[c]	
HLA	Allele	Percentage of population	Allele	Percentage of population	Allele	Percentage of population
HLA-A	0201[d,e]	30	0201[f]	41	0201[f]	45
	2402[d,e]	27	0101[f]	23	0101[f]	27
	0301	17	0301	21	0301	22
	0101[d,e]	10	2402[f]	17	2402[f]	17
	1101[e]	5	1101	10	1101[f]	15
HLA-B	4002[d,e]	19	0702	21	0702	21
	0702	14	4402[f]	19	0801[f]	20
	4402[d,e]	9	0801[f]	16	4402[f]	16
	3501	11	3501	13	4403	11
	5301[e]	8	1501[f]	12	3501	11
	4403	8	4403	8	4001[f]	11
	5101	6	4001[f]	8	5101	10
	0801[d,e]	3	5101	6	1501[f]	7
	4001[d,e]	2	5301[e]	4	4002[f]	1
	1501[d,e]	0	4002[f]	2	5301[d,f]	1
HLA-CW	0401[e]	32	0401[e]	26	0701[f]	27
	0702	21	0701[f]	26	0702	23
	0602[e]	12	0702	23	0602[f]	21
	0202	10	0602	20	0401[d,f]	19
	0501[d,e]	9	0501[f]	18	0501[f]	19
	0305[d,e]	9	0304	11	0304	13
	0102	8	0202	8	0202	9
	0304	8	0102	5	0102	7
	0701[d,e]	5	0305[f]	0	0305[f]	0

NOTE. The alleles listed have frequencies ⩾5% in at least one study control population.

[a] Control subjects were typed by loci: *HLA-A* (n = 112), *HLA-B* (n = 102), and *HLA-CW* (n = 95).

[b] Control subjects were typed by loci: *HLA-A* (n = 203), *HLA-B* (n = 208), and *HLA-CW* (n = 206).

[c] Control subjects were typed by loci: *HLA-A* (n = 333), *HLA-B* (n = 322), and *HLA-CW* (n = 330).

[d] The allele frequency was statistically significantly different (χ^2 test) from the eastern US study control population.

[e] The allele frequency was statistically significantly different (χ^2 test) from the Portland study control population.

[f] The allele frequency statistically was significantly different (χ^2 test) from the Costa Rican study control population.

timates were adjusted by study (each study as a dummy variable) to account for potential differences among the 3 populations.

We calculated the Pearson correlation coefficients between alleles to identify alleles in possible linkage disequilibrium (LD; defined as $r \geqslant 0.9$). Although LD could only be inferred on the basis of a high correlation coefficient, this was done to ensure that independent associations between these alleles and disease could be calculated. To identify HLA alleles associated with disease progression, we conducted HPV-restricted analyses in which patients with cancer or HSILs were compared with patients with LSILs and HPV-positive control subjects, thus identifying HLA class I alleles associated with progression from low-grade to high-grade disease. In addition, we conducted HPV-16–restricted analyses to determine whether HPV type specificity for class I allele-disease associations existed, as shown for class II alleles [4]. The HPV-16–restricted analysis was conducted in the Portland cohort because of the study design, which oversampled HPV-16–positive control subjects; however, these analyses were not done for the eastern US and Costa Rican studies because of the minimal number of HPV-16–positive population control subjects. Statistical analyses were done with SAS software (version 8.12; SAS Institute). All tests of statistical significance are 2-sided.

Results

Allele frequencies (by individual subjects) in the 3 population control groups were first compared. Alleles with frequencies of ⩾5% in any study control group are summarized in table 2. As expected, statistically significant differences were observed mostly between the Costa Rican admixed population and the 2 predominantly white US populations. For example, although *HLA-B*4002* had a relatively high allele frequency of 19% among the Costa Rican control population, its allele frequency was 2% among the eastern US study control population and 1% among the Portland control population. In addition, some alleles of relatively high frequency in the US studies (e.g., *HLA-CW*0701* at 26% in the eastern US study and 27% in the Portland study) had low allele frequencies in Costa Rica (5%). Even consistently high-frequency alleles, such as *HLA-A*0201,* were statistically significantly different between the studies (30% in Costa Rica, 41% in the eastern US study, and 45% in the Portland study). Nevertheless, a number of allele frequencies were consistent across all 3 populations (e.g., *HLA-B*3501* at 11%–13%, *CW*0702* at 21%–26%, and *HLA-CW*0202* at 8%–

10%). Alleles that are statistically significantly different from the other 2 study populations are indicated in table 2.

The allele frequency among patients with cancer or HSILs and those with LSILs was significantly different ($P < .05$) from that of control subjects in any one study or in combined analyses for the following alleles: *HLA-A*0206, A*0301, A*3101, A*3103, A*3303, A*3402, A*6803, B*1402, B*1508, B*1512, B*2705, B*3503, B*3517, B*3901, B*3908, B*4901, B*5301, B*8101, CW*0202, CW*0305, CW*0401, CW*0801,* and *CW*0802*. For these alleles, we conducted further analyses. First, we calculated the Pearson correlation coefficients for these alleles with each other and with all other HLA class I alleles. No combination with these alleles had a correlation coefficient of $r \geq 0.9$, denoting possible LD, nor were any of these alleles moderately correlated. We therefore proceeded to assess independent associations between these alleles with disease.

Table 3 shows the disease ORs for these alleles and for cancer or HSILs by study. For a single allele, *HLA-CW*0202,* the allele frequency among patients with cancer or HSILs was significantly lower than that among control subjects (merged OR, 0.53; 95% CI, 0.29–0.89). This risk decrease was consistent across all 3 studies with an OR of 0.75 (95% CI, 0.35–1.62) in Portland, 0.22 (95% CI, 0.06–0.80) in Costa Rica, and 0.62 (95% CI, 0.24–1.63) in the eastern United States. Other alleles that appeared to decrease the risk for cancer or HSILs were *HLA-B*2705* and *HLA-CW*0401*. However, although a statistically significant decrease in risk for cancer or HSILs was observed for both alleles in the eastern US study (*HLA-B*2705:* OR, 0.29; 95% CI, 0.08–0.99; *HLA-CW*0401:* OR, 0.52; 95% CI, 0.29–0.94), the decreases in risk were not statistically significant in Costa Rica and they were not observed in the Portland study.

No single allele had a statistically significant or consistent increase in risk for cancer or HSILs. Although possession of *HLA-B*1508* appeared to increase the risk for cancer or HSILs in all 3 studies, the risk estimates were not statistically significant in the studies or in the merged analyses (OR, 9.31; 95% CI, 0.79–58.5). Furthermore, although possession of the *HLA-A*3303, -B*3503,* or *-B*3901* alleles statistically significantly increased the risk for cancer or HSILs in the Portland study, with risk estimates of 3.35 (95% CI, 1.26–8.88), 2.95 (95% CI, 1.08–8.03), and 3.43 (95% CI, 1.13–10.4), respectively, the increases in risk were not observed in the Costa Rican or eastern US study.

Table 4 shows the disease ORs for alleles and LSILs. The eastern US study was not included in this analysis because it did not include patients with LSILs. Consistent with findings for patients with cancer or HSILs, *HLA-CW*0202* was associated with a decreased risk for developing LSILs in the merged analyses (OR, 0.58; 95% CI, 0.37–1.04) and by study (Portland: OR, 0.52; 95% CI, 0.25–1.07; Costa Rica: OR, 0.73; 95% CI, 0.26–2.02), albeit not statistically significant. In addition, although possession of the *HLA-B*4901* allele consistently reduced the risk for developing LSILs in Portland (OR, 0.48; 95% CI, 0.13–1.74) and Costa Rica (OR, 0.37; 95% CI, 0.04–3.33), the risk estimates were not statistically significant nor were they statistically significant in merged analyses (OR, 0.45; 95% CI, 0.15–1.36).

Table 3. Association between HLA class I alleles and cancer/high-grade squamous epithelial lesions, by study.

	Portland, Oregon				Costa Rica				Eastern United States			
HLA allele	No. (%) of case patients (n = 124)	No. (%) of control subjects (n = 350)	OR	95% CI	No. (%) of case patients (n = 124)	No. (%) of control subjects (n = 118)	OR	95% CI	No. (%) of case patients (n = 117)	No. (%) of control subjects (n = 213)	OR	95% CI
*A*0206*	0	0	—	—	7 (5.7)	6 (5.1)	1.12	0.36–3.43	1 (0.9)	0	—	—
*A*0301*	31 (25)	78 (22)	1.16	0.72–1.88	19 (15)	20 (17)	0.89	0.45–1.76	28 (24)	45 (21)	1.17	0.69–2.01
*A*3101*	6 (4.8)	11 (3.1)	1.57	0.57–4.33	8 (6.5)	8 (6.8)	0.95	0.34–2.61	3 (2.5)	8 (3.8)	0.67	0.18–2.59
*A*3103*	0	0	—	—	0	0	—	—	0	0	—	—
*A*3303*	9 (7.3)	8 (2.3)	3.35	1.26–8.88	3 (2.4)	2 (1.7)	1.44	0.24–8.76	2 (1.7)	4 (1.9)	0.91	0.16–5.04
*A*3402*	2 (1.6)	0	—	—	1 (0.8)	4 (3.4)	0.23	0.03–2.10	1 (0.9)	4 (1.9)	0.45	0.05–4.08
*A*6803*	0	1 (0.3)	—	—	9 (7.3)	5 (4.2)	1.77	0.58–5.44	2 (1.7)	0	—	—
*B*1402*	4 (3.2)	10 (29)	1.13	0.35–3.68	10 (8.1)	4 (3.4)	2.50	0.76–8.20	2 (1.7)	8 (3.8)	0.45	0.09–2.13
*B*1508*	2 (1.6)	0	—	—	2 (1.6)	0	—	—	1 (0.9)	1 (0.5)	1.83	0.11–29.5
*B*1512*	0	1 (0.3)	—	—	0	0	—	—	0	0	—	—
*B*2705*	12 (9.7)	28 (8.0)	1.23	0.61–2.51	2 (1.6)	2 (2.5)	0.63	0.10–3.83	3 (2.6)	18 (8.5)	0.29	0.08–0.99
*B*3503*	8 (6.5)	8 (2.3)	2.95	1.08–8.03	3 (2.4)	3 (2.5)	0.95	0.19–4.81	6 (5.1)	6 (2.8)	1.89	0.59–5.92
*B*3517*	0	0	—	—	0	3 (2.4)	—	—	0	0	—	—
*B*3901*	7 (5.7)	6 (1.7)	3.43	1.13–10.4	1 (0.8)	1 (0.9)	0.85	0.06–15.4	1 (0.8)	7 (3.3)	0.25	0.03–2.19
*B*3908*	0	0	—	—	7 (5.7)	4 (3.4)	1.71	0.49–5.98	0	0	—	—
*B*4901*	4 (3.2)	11 (3.1)	1.03	0.32–3.29	3 (2.4)	4 (3.4)	0.71	0.16–3.23	5 (4.3)	15 (7.0)	0.59	0.21–1.67
*B*5301*	1 (0.8)	3 (0.9)	0.94	0.10–9.13	6 (4.8)	10 (8.5)	0.55	0.19–1.56	0	8 (3.8)	—	—
*B*8101*	2 (1.6)	0	—	—	0	1 (0.9)	—	—	0	0	—	—
*CW*0202*	9 (7.3)	33 (9.4)	0.75	0.35–1.62	3 (2.4)	12 (10.2)	0.22	0.06–0.80	6 (5.1)	17 (8.0)	0.62	0.24–1.63
*CW*0305*	0	0	—	—	12 (9.7)	11 (9.3)	1.04	0.44–2.46	1 (0.9)	0	—	—
*CW*0401*	27 (22)	67 (19)	1.18	0.71–1.94	37 (30)	38 (32)	0.90	0.52–1.54	18 (15)	55 (26)	0.52	0.29–0.94
*CW*0801*	2 (1.6)	0	—	—	0	0	—	—	1 (0.9)	1 (0.5)	1.83	0.11–29.5
*CW*0802*	6 (4.8)	25 (7.1)	0.66	0.27–1.65	9 (7.3)	6 (5.1)	1.46	0.50–4.24	5 (4.3)	13 (6.1)	0.69	0.24–1.98

NOTE. CI, confidence interval; OR, odds ratio.

Table 4. Association between HLA class I alleles and low-grade squamous squamous epithelial cells, by study.

HLA allele	Portland, Oregon				Costa Rica			
	No. (%) of case patients (n = 196)	No. (%) of control subjects (n = 350)	OR	95% CI	No. (%) of case patients (n = 78)	No. (%) of control subjects (n = 118)	OR	95% CI
*A*0206*	0	0	—	—	7 (8.9)	6 (5.1)	1.82	0.59–5.62
*A*0301*	54 (28)	78 (22)	1.33	0.89–1.98	9 (11)	20 (17)	0.63	0.27–1.47
*A*3101*	14 (7.1)	11 (3.1)	2.27	1.05–5.33	8 (10)	8 (6.8)	1.55	0.56–4.32
*A*3103*	2 (1.0)	0	—	—	0	0	—	—
*A*3303*	5 (2.6)	8 (2.3)	1.12	0.36–3.47	0	2 (1.7)	—	—
*A*3402*	0	0	—	—	2 (2.5)	4 (3.4)	0.74	0.13–4.14
*A*6803*	0	1 (0.3)	—	—	4 (5.1)	5 (4.2)	1.21	0.31–4.64
*B*1402*	7 (3.6)	10 (2.9)	1.26	0.47–3.36	9 (11)	4 (3.4)	3.66	1.09–12.3
*B*1508*	0	0	—	—	0	0 (1.6)	—	—
*B*1512*	5 (2.6)	1 (0.3)	9.14	1.06–78.8	0	0	—	—
*B*2705*	12 (6.1)	28 (8.0)	0.75	0.37–1.51	0	3 (2.5)	—	—
*B*3503*	6 (3.1)	8 (2.3)	1.35	0.46–3.95	1 (1.3)	3 (2.5)	0.49	0.05–4.81
*B*3517*	1 (0.5)	0	—	—	0	3 (2.4)	—	—
*B*3901*	5 (2.6)	6 (1.7)	1.50	0.45–4.98	1 (1.3)	1 (0.9)	1.50	0.09–24.3
*B*3908*	0	0	—	—	6 (7.6)	4 (3.4)	2.34	0.64–8.59
*B*4901*	3 (1.5)	11 (3.1)	0.48	0.13–1.74	1 (1.3)	3 (3.4)	0.37	0.04–3.33
*B*5301*	5 (2.6)	3 (0.9)	3.03	0.72–12.8	1 (1.3)	10 (8.5)	0.14	0.02–1.10
*B*8101*	0	0	—	—	0	1 (0.9)	—	—
*CW*0202*	10 (5.1)	33 (9.4)	0.52	0.25–1.07	6 (7.6)	12 (10.2)	0.73	0.26–2.02
*CW*0305*	0	0	—	—	10 (13)	11 (9.3)	1.41	0.57–3.50
*CW*0401*	40 (20)	67 (19)	1.09	0.70–1.68	19 (24)	38 (32)	0.67	0.35–1.27
*CW*0801*	1 (0.5)	0	—	—	1 (1.3)	0	—	—
*CW*0802*	20 (10)	25 (7.1)	1.48	0.80–2.74	10 (13)	6 (5.1)	2.71	0.94–7.77

NOTE. CI, confidence interval; OR, odds ratio.

A statistically significant increase in risk for LSILs in the merged analyses was observed for *HLA-A*3101* (OR, 2.02; 95% CI, 1.07–3.80), which was consistent in Portland (OR, 2.27; 95% CI, 1.05–5.33) and Costa Rica (OR, 1.55; 95% CI, 0.56–4.32). An increase in risk for developing LSILs in the merged analyses was also observed for *HLA-CW*0802* (OR, 1.73; 95% CI, 1.02–2.93), with study-specific ORs of 1.48 (95% CI, 0.80–2.74) in Portland and 2.71 (95% CI, 0.94–7.71) in Costa Rica. These increases in risk observed for LSILs *(HLA-A*3101* and *-B*0802)* were not observed for patients with cancer or HSILs.

Although possession of the *HLA-B*1512* allele also appeared to increase risk for LSILs, this allele was only present in the Portland population. In addition, although not statistically significant in the merged analyses (OR, 1.95; 95% CI, 0.94–4.07), possession of the *HLA-B*1402* allele did statistically significantly increase risk for LSILs in Costa Rica (OR, 3.66; 95% CI, 1.09–12.3). This finding was consistent, but not statistically significant, in the Portland study (OR, 1.26; 95% CI, 0.47–3.36).

To evaluate the association between HLA class I alleles and HPV progression, we conducted analyses restricted to HPV-infected women (table 5). Thus, we compared women with cancer or HSILs with those with LSILs and HPV-positive control women and elevated the baseline group to women at risk for progression. Findings significant in merged analyses included a decreased risk for *HLA-CW*0802* (OR, 0.52; 95% CI, 0.28–0.96), which was consistent, albeit not statistically significant, across the studies (Portland: OR, 0.51; 95% CI, 0.21–1.26; Costa Rica: OR, 0.57; 95% CI, 0.21–1.57; eastern US: OR, 0.42; 95% CI, 0.10–1.86). In addition, although possession of *HLA-B*3901* did not increase risk for progression in the Costa Rica study, the risk estimate was significant in the Portland study (OR, 3.48; 95% CI, 1.15–10.6). Likewise, possession of *HLA-CW*0401* statistically significantly decreased risk for progression only in the eastern US study (OR, 0.26; 95% CI, 0.10–0.72). Finally, although possession of *HLA-CW*0202* appeared to decrease risk in merged analyses (OR, 0.68; 95% CI, 0.35–1.31) and in all 3 individual studies, none was statistically significant.

Only in the Portland study were we able to conduct analyses to assess HPV-16 type specificity, because of the original study design, which oversampled control subjects among HPV-16–positive women. For this population, none of the previously mentioned alleles had statistically significant allele-disease associations and thus did not support HPV-16 type specificity for these associations (data not shown). The association for *HLA-CW*0202* with HPV-16–positive patients with HSILs, compared with HPV-16–positive control subjects, in merged analysis was 0.93 (95% CI, 0.41–2.11); for *HLA-CW*0802,* the merged risk estimate was 0.68 (95% CI, 0.26–1.75).

Discussion

We found a reduction in disease risk with a single allele, *HLA-CW*0202*. Unlike other alleles examined, the association between *HLA-CW*0202* and cancer or HSILs was statistically

Table 5. Association between HLA class I alleles and cancer/high-grade squamous intraepithelial lesions, by study, restricted to human papillomavirus–infected women.

HLA allele	Portland, Oregon				Costa Rica				Eastern United States			
	No. (%) of case patients (n = 124)	No. (%) of control subjects (n = 355)	OR	95% CI	No. (%) of case patients (n = 111)	No. (%) of control subjects (n = 95)	OR	95% CI	No. (%) of case patients (n = 60)	No. (%) of control subjects (n = 45)	OR	95% CI
*A*0206*	0	0	—	—	7 (6.3)	7 (7.4)	0.85	0.29–2.50	0	0	—	—
*A*0301*	31 (25)	91 (26)	0.07	0.60–1.55	18 (16.2)	10 (10.5)	1.65	0.72–3.76	13 (22)	7 (16)	1.50	0.55–4.14
*A*3101*	6 (4.8)	19 (5.4)	0.90	0.35–2.31	7 (6.3)	10 (10.5)	0.57	0.21–1.57	1 (1.7)	2 (4.4)	0.36	0.03–4.15
*A*3103*	0	2 (0.6)	—	—	0	0	—	—	0	0	—	—
*A*3303*	9 (7.3)	9 (2.5)	3.00	1.17–7.76	2 (1.8)	0	—	—	1 (1.7)	1 (2.2)	0.75	0.05–12.3
*A*3402*	2 (1.6)	0	—	—	1 (0.9)	2 (2.1)	0.42	0.04–4.74	0	0	—	—
*A*6803*	0	1 (0.3)	—	—	9 (8.1)	4 (4.2)	2.00	0.60–6.74	0	0	—	—
*B*1402*	4 (3.2)	13 (3.7)	0.88	0.28–2.74	8 (7.2)	9 (9.5)	0.74	0.28–2.01	1 (1.7)	1 (2.2)	0.75	0.05–12.3
*B*1508*	2 (1.6)	0	—	—	1 (0.9)	0	—	—	1 (1.7)	0	—	—
*B*1512*	0	6 (1.7)	—	—	0	0	—	—	0	0	—	—
*B*2705*	12 (9.7)	24 (6.8)	1.48	0.72–3.05	2 (1.8)	0	—	—	2 (3.3)	5 (11.1)	0.28	0.05–1.49
*B*3503*	8 (1.7)	11 (3.1)	2.16	0.85–5.50	3 (2.7)	2 (2.1)	1.29	0.21–7.90	2 (3.3)	2 (4.4)	0.74	0.10–5.47
*B*3517*	0	1 (0.3)	—	—	2 (1.8)	0	—	—	0	0	—	—
*B*3901*	7 (5.7)	6 (1.7)	3.48	1.15–10.6	1 (0.9)	1 (1.1)	0.85	0.15–13.8	0	0	—	—
*B*3908*	0	0	—	—	7 (6.3)	6 (6.3)	1.00	0.32–3.08	0	0	—	—
*B*4901*	4 (3.2)	9 (2.5)	1.28	0.39–4.24	3 (2.7)	1 (1.1)	2.61	0.27–25.5	3 (5.0)	2 (4.4)	1.13	0.18–7.07
*B*5301*	1 (0.8)	7 (2.0)	0.40	0.05–3.32	5 (4.5)	1 (1.1)	4.43	0.51–38.6	0	3 (6.7)	—	—
*B*8101*	2 (1.6)	0	—	—	0	0	—	—	0	0	—	—
*CW*0202*	9 (7.3)	26 (7.3)	0.99	0.45–2.18	2 (1.8)	7 (7.4)	0.23	0.05–1.14	3 (5.0)	3 (6.7)	0.74	0.14–3.83
*CW*0305*	0	0	—	—	11 (9.9)	12 (12.6)	0.76	0.32–1.81	0	0	—	—
*CW*0401*	27 (22)	74 (21)	1.06	0.64–1.74	35 (31.5)	24 (25.3)	1.36	0.74–2.51	7 (12)	15 (14)	0.26	0.10–0.72
*CW*0801*	2 (1.6)	1 (0.3)	5.80	0.52–64.5	0	1 (1.1)	—	—	0	1 (2.2)	—	—
*CW*0802*	6 (4.8)	32 (9.0)	0.51	0.21–1.26	7 (6.3)	10 (10.5)	0.57	0.21–1.57	3 (5.0)	5 (11)	0.42	0.10–1.86

NOTE. CI, confidence interval; OR, odds ratio.

significant in combined analyses and was consistent across all 3 populations and in the 2 predominant ethnic groups (admixture in Costa Rica and whites in the United States). Although not reaching statistical significance for the individual US studies, the decrease in risk for cancer or HSILs was statistically significant in the Costa Rican study, where the prevalence of *HLA-CW*0202* was also the highest (10%). This association was also consistent, albeit not statistically so, for LSILs in merged and study-specific analyses. These findings support the hypothesis that possession of a single protective HLA allele is sufficient for protection from HPV infection and subsequent cervical neoplasia.

*HLA-CW*0802* was associated with a decreased risk for disease progression but was associated with an increased risk for LSILs (with no association observed for cancer or HSILs). We also found an increased risk for LSILs for women with *HLA-A*3101* but not for cancer or HSILs. From the current analyses, it is not clear why these alleles might increase risk for LSILs but not for HSILs/cancer or why *HLA-CW*0802* might simultaneously be associated with an increased risk for LSILs but a decreased risk for progression. From our analyses, it is difficult to conclude whether these differential associations indicate potentially different roles for each allele (e.g., in initial infection by HPV or immunosurveillance of infected cells). In addition to a chance finding, another possible explanation may reside in the unmeasured alleles with which these alleles are in LD. On the basis of the current data, it is difficult to draw conclusions about the roles that *HLA-CW*0802* and *HLA-A*3101* play in cervical neoplasia.

Limitations in the present study include the potential for confounding by ethnicity, also known as population stratification [24], in merged analysis. Although we accounted for potential confounding due to population stratification by adjusting for study in our merged analyses, the theoretical possibility of residual confounding within studies remains. However, we believe that within-study confounding by ethnicity is not likely to strongly affect our results because, in Costa Rica, the population is highly admixed, and, in our US-based studies, the vast majority of subjects were of white ethnicity. Limitations with merged analyses also include the potential for missing associations that may exist if LD with another allele (whether HLA or another critical gene) is needed for an association to be present. As alluded to earlier, it is plausible that inconsistent findings between the different studies may be real and due to possible LD with another critical allele that we have not measured and which may not be present in another population; such associations would not be detected in our present analyses.

Future directions would include identification of complete HLA class I and II haplotypes and assessing their relationships with disease. It is also plausible that some of the inconsistent findings in this study may be attributed to false-positive results from the multiple comparisons of HLA alleles. Because of these limitations, criteria for significance consisted not only of statistical significance in one study or in merged analysis but also

of consistent findings across studies, in addition to statistical significance in the merged analysis. Nevertheless, although associations consistent in all 3 studies benefited from the statistical significance gained by merging the studies, the lack of statistical significance within the individual studies remains a limitation.

Strengths of our study included extensive high-resolution HLA genotyping by one laboratory. This allowed identification of individual allele-disease associations in HLA class I alleles, which has not been reported previously. Furthermore, our findings are from 3 large independent studies plus analyses that merged all 3 studies when results were consistent across studies. Merging the different studies enhanced the sample size for assessing individual allele-disease associations plus data from 3 studies provided sufficient strength to ensure consistency in findings across populations and ethnic groups. We emphasize that this ability to identify consistency across populations and ethnic groups is the real strength of the present analyses. For alleles with adequate frequencies in each study (~5% frequency in control subjects), such as for *HLA-CW*0202,* the pooling of data provided robust analyses. For alleles with widely varying frequencies between studies, we emphasize the need for consistent findings across individual studies. This need is due to the unique control definitions for each study; although all control subjects are defined as cytologically normal, the Costa Rican and eastern US study control subjects were population based, whereas the Portland study control subjects were oversampled for HPV-16–positive women.

As noted, the importance of HLA class I molecules in cervical neoplasia is well established. Down-regulation of HLA class I antigens affects immune surveillance of viral infection and affects effective elimination of infected cells [25, 26]. For other immune-related disorders, such as ankylosing spondylitis, psoriasis, and AIDS progression, HLA class I allele–disease associations have been established. To our knowledge, our study is the first to comprehensively assess HLA class I alleles and cervical neoplasia. The observation consistent in all 3 studies and across the 2 ethnic groups is the decreased risk for developing cancer or HSILs and LSILs observed for women with the *HLA-CW*0202* allele. However, some women with cancer or HSILs possess the *HLA-CW*0202* allele, suggesting that the importance of additional factors involved in the disease process and emphasizing that the role of HLA molecules is only one of many factors involved in disease development. Future studies should include the complete assessment of HLA class I and class II molecules (haplotype analyses), assessment of HPV type specificity (to identify protective or risk alleles specific to oncogenic HPV types), and the exploration of innate immunity.

On the basis of the present results, we believe that an exploration of the role of NK cells is warranted because of the role HLA-C molecules play in stimulating cellular immune responses via their recognition by NK cells [27, 28]. It is plausible that the protective association observed for disease and disease progression with HLA-C alleles is indicative of the involvement that CTL and NK cells play in the host response to viral infections and in recognizing and destroying human tumor cells [28]. The lack of HPV type specificity observed for *HLA-CW*0202* with disease is also consistent with a proposed role for NK cells, since NK cell responses are not antigen specific. We suggest there may be a possible involvement of NK function in HPV infection and subsequent cervical neoplasia.

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